BPB Reports

Report

Diet-induced Differences in the Gut Microbiome and Mycobiome in Conventionally Housed Mice

Kento Takayama,[a,b,*] Motoya Miyahara,[a,b] Nana Morio-Takayama,[b] Mairi Fujikawa,[b] Honoka Ito,[a] Yuka Koike,[a] and Kazuyoshi Kawazoe[a]

[a]*School of Pharmacy, Showa Medical University, 1-5-8 Hatanodai Shinagawa-ku, Tokyo 142-8555, Japan;* [b]*Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, 1 Sanzo, Gakuen-cho, Fukuyama, Hiroshima 729-0292, Japan*

Received February 13, 2026; Accepted October 26, 2026

The human gut hosts diverse microbes essential for metabolism, immune function, and disease prevention. Imbalance, or dysbiosis, of the gut microbiota contributes to various diseases, with diet exerting a stronger influence than genetics. We investigated the effects of five diets, chow, high-carbohydrate, high-fat, high-protein, and high-fiber, on the gut microbiota (bacteria) and mycobiota (fungi) in conventionally housed mice, highlighting implications for pharmaceutical research. Gut microbial composition varied with diet: the phylum Bacillota dominated in the chow, high-carbohydrate, and high-fat diet groups, whereas Bacteroidota predominated in the high-protein and high-fiber diet groups. Linear Discriminant Analysis Effect Size analysis identified distinct bacterial taxa enriched in each dietary group compared with chow. Fungal analysis showed a shift from Ascomycota dominance in chow to Basidiomycota in the other diet groups. Correlation analysis indicated that bacterial–fungal relationships varied with diet; notably, the high-protein diet group showed numerous strong negative correlations between bacterial and fungal families. These findings emphasize that diet shapes microbial composition and interactions in animal models, providing baseline information for considering diet-associated microbial variation in preclinical research.

Key words diet, gut microbe, gut microbiome, gut mycobiome, bacteria-fungi correlation

INTRODUCTION

The human gut harbors approximately 40 trillion microorganisms, comprising up to 1000 bacterial species, collectively referred to as the gut microbiota, which supports host health by producing essential metabolites, regulating bile acid metabolism, detoxifying harmful compounds, and maintaining intestinal barrier function.[1)] Dysbiosis, defined as qualitative and quantitative imbalances in the gut microbiota, is associated with both intestinal and systemic diseases, highlighting the importance of restoring microbial balance for disease prevention.[2–4)] Accordingly, growing interest has been shown in probiotics and prebiotics that promote gut microbial growth.

Host genetics appear to play a limited role in shaping gut microbiota composition. Rothschild *et al*. demonstrated that interindividual differences are primarily driven by lifestyle factors such as diet and drug exposure.[5)] In pharmaceutical research, the gut microbiome is increasingly recognized as influencing drug efficacy and toxicity; for example, Nagata *et al.* developed a drug–microbe interaction catalog for microbiome-mediated drug metabolism.[6)] We previously reported that Daiokanzoto (Da-Huang-Gan-Cao-Tang), a traditional Japanese medicine used for constipation, exerts purgative effects by enhancing gut microbial enzymes responsible for sennoside A metabolism.[7–10)] Furthermore, dietary composition and antibiotic treatment alter the gut microbiota, thereby affecting the intestinal metabolism and laxative efficacy of Daiokanzoto.[11,12)]

Although bacteria constitute the majority of gut microorganisms, fungi account for approximately 0.1% of the gut microbial population.[13,14)] Since the introduction of the "mycobiome" concept in 2009,[15)] their biological significance has become increasingly apparent. Gut fungi produce metabolites that influence bacterial growth and the overall gut ecosystem.[16)] Fungal communities also play key roles in shaping host immune responses, and mycobiome dysbiosis has been implicated in inflammatory arthritis, pseudomembranous colitis, and multiple sclerosis.[17–20)]

Dietary composition is also an important experimental variable in animal studies involving the gut microbiome. Previous studies have demonstrated that differences in rodent diets can substantially alter gut bacterial community structure and microbial fermentation profiles, potentially contributing to variability in preclinical studies.[21)] Furthermore, dietary inter-

*To whom correspondence should be addressed. e-mail: kento.takayama@showa-u.ac.jp

© 2026 The Author(s) BPB Reports applies the Creative Commons Attribution (CCBY) license to works we published. The license was developed to facilitate open access - namely, free immediate access to, and unrestricted reuse of, original works to all types. Under this license, authors agree to make articles legally available for reuse, without permissions of fees, for virtually any purpose. Anyone may copy, distribute, or reuse these articles, as long as the author and original source are properly cited. https://creativecommons.org/licenses/by/4.0/

ventions have been shown to affect not only bacterial communities but also the gut mycobiome and bacteria–fungi relationships in mice.[22,23] Thus, characterization of the microbial background established under different dietary conditions may be important for the interpretation and reproducibility of preclinical studies.

Given the potential interactions between drugs and gut microbes, understanding how dietary conditions shape the microbial background of experimental animals may be relevant to the interpretation of pharmacological studies. Therefore, the present study aimed to establish baseline microbial profiles under five dietary conditions with different macronutrient compositions. We compared both the gut bacterial microbiome and mycobiome and further examined bacteria–fungi correlations within each dietary condition.

MATERIALS AND METHODS

Animal Preparation Animal experiments followed the Guidelines for Animal Experimentation of Fukuyama University. Male C57BL/6N mice (20–30 g; Shimizu Laboratory Supplies, Kyoto, Japan) were housed under a 12-h light/dark cycle at 21–24°C for at least 1 week before experimentation. Mice had ad libitum access to the chow diet and water during the 1-week acclimatization period. After acclimatization, the 30 mice were allocated to five cages (six mice per cage) based on body weight so that the mean body weight was comparable among cages. Each cage was then assigned to one of five dietary conditions (one cage per diet) and fed the respective diet for 28 d: chow, high-carbohydrate, high-fat, high-protein, or high-fiber. The chow diet (CLEA Rodent Diet CE-2) was purchased from CLEA Japan, Inc. (Tokyo, Japan). The macronutrient compositions of the purified diets, expressed as percentages of total energy (kcal%), were 20% protein, 70% carbohydrate, and 10% fat for the high-carbohydrate diet (D12450H); 20% protein, 35% carbohydrate, and 45% fat for the high-fat diet (D12451); and 50% protein, 40% carbohydrate, and 10% fat for the high-protein diet (D15122105). The high-fiber diet (D14053101) was based on the high-carbohydrate diet and contained 117 g of inulin in addition to 50 g of cellulose. For the chow diet (CE-2), the manufacturer-reported proximate composition is provided in Supplementary Table 1 because detailed ingredient amounts and caloric values are not publicly disclosed. All diets except chow were obtained from Research Diets, Inc. (New Brunswick, NJ, USA).

Sample Collection and DNA Extraction Fecal samples were collected on day 28. Approximately 100 mg of feces per mouse was mechanically lysed using a Micro Smash MS-100 (Tomy Seiko Co., Ltd., Tokyo, Japan), and total DNA was extracted using phenol/chloroform/isoamyl alcohol, precipitated, washed with ethanol, resuspended in Tris-EDTA buffer containing RNase A (Sigma-Aldrich, St. Louis, MO, USA), and further purified using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) before suspension in 200 μL of elution buffer.

Analysis of Intestinal Microbiome Composition The V1–V2 region of the bacterial 16S rRNA gene was amplified using primers 27F-mod (5′-AGRGTTTGATYMTGGCTCAG-3′) and 338R (5′-TGCTGCCTCCCGTAGGAGT-3′) each attached to forward (5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNN-3′) and reverse (5′-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNN-3′) adapter sequences, respectively. The internal transcribed spacer 1 (ITS1) region of the fungal rRNA gene was amplified using primers ITS1Fngs (5′-GGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′), each with the forward (5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3′) and reverse (5′-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3′) adapters, respectively. PCR amplification was performed using Tks Gflex DNA Polymerase (Takara Bio, Shiga, Japan). PCR products were purified using the AMPure XP Kit (Beckman Coulter, Brea, CA, USA) and indexed using primers 2ndF (5′-AATGATACGGCGACCACCGAGATCTACAC-Index2-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3′) and 2ndR (5′-CAAGCAGAAGACGGCATACGAGAT-Index1-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3′). The indexed libraries were purified and quantified using a Qubit 4 Fluorometer (Thermo Fisher Scientific K.K., Tokyo, Japan), pooled at equimolar concentrations, and sequenced on an Illumina MiSeq platform (MiSeq Reagent Kit v3, 600 cycles) with PhiX control v3 (Illumina, San Diego, CA, USA). Sequence data were processed using QIIME2 (version 2021.4).[24] Primer sequences were removed with the Cutadapt plugin,[25] and reads were denoized and clustered into amplicon sequence variants (ASVs) using DADA2.[26] Taxonomic classification was performed against the SILVA 138 database[27] for 16S rRNA and the UNITE 8.0 database[28] for ITS sequences. The sequencing depth was 29841 ± 9947 reads/sample (mean ± SD) for bacterial 16S rRNA gene sequencing and 39674 ± 18802 reads/sample (mean ± SD) for fungal ITS sequencing. Prior to α-diversity analysis, the sequencing data were rarefied to 10,000 reads per sample. ASVs were used to calculate α-diversity metrics (observed features, Faith's phylogenetic diversity, Shannon index) and β-diversity metrics based on unweighted UniFrac distances. Visualization was performed in R (version 4.1.2),[29] using ggplot2, gridExtra, and reshape2. Linear Discriminant Analysis Effect Size (LEfSe) was conducted via the Galaxy platform (http://huttenhower.sph.harvard.edu/galaxy),[30] with a logarithmic LDA score threshold of 2.0.

Statistical Analysis Data are presented as the mean ± SD. α-diversity differences were evaluated using the nonparametric Kruskal–Wallis test followed by Steel's test (Kyplot 5.0, KyensLab Inc.). β-diversity was visualized using principal coordinate analysis (PCoA) and differences in microbial community structure were assessed via permutational multivariate analysis of variance (PERMANOVA). For the correlation analysis, bacterial and fungal families accounting for ≥0.1% of the total bacterial or fungal community, respectively, in at least one dietary group were included, resulting in 22 bacterial families and 23 fungal families. Correlations between bacterial and fungal communities were calculated using Spearman's rank correlation. Statistical significance was defined as $p < 0.05$.

Ethical Approval All experimental protocols involving mice were approved by the Research Ethics Committee of Fukuyama University and conducted in accordance with its Guidelines for Animal Experimentation (approval number: 2021-A-2).

RESULTS

Effect of Diet on α-Diversity of Gut Microbiome and Mycobiome Analysis of gut microbiome α-diversity revealed that species richness (number of bacterial species) under 10 000 reads ranged between 100 and 300 species (Fig. 1A). Observed features were reduced by approximately 50% in the high-carbohydrate ($p = 0.036$), high-protein ($p = 0.014$), and high-fiber ($p = 0.014$) groups, with the lowest richness in high-fiber. The Shannon index (species evenness) was also significantly lower in the high-carbohydrate ($p = 0.036$) and high-fiber ($p = 0.023$) diet groups compared with chow. In contrast, the gut mycobiome showed an estimated richness of 80–160 fungal species (Fig. 1B), with no significant differences in richness or evenness across groups.

These results suggest that diet strongly influences bacterial diversity in the gut microbiome, whereas the mycobiome remains relatively stable.

Effect of Diet on β-Diversity of Gut Microbiome and Mycobiome Analysis of β-diversity revealed that gut microbiome communities formed significantly distinct clusters across all pairwise dietary comparisons ($p < 0.01$; Fig. 2A). Similarly, the gut mycobiome showed distinct clustering in the chow and high-fiber groups compared with other dietary groups (Fig. 2B). Pairwise comparisons indicated significant differences between chow and high-carbohydrate ($p = 0.001$), chow and high-fat ($p = 0.004$), chow and high-protein ($p = 0.005$), chow and high-fiber ($p = 0.002$), and between high-fiber and high-carbohydrate ($p = 0.001$), high-fat ($p = 0.002$), and high-protein ($p = 0.002$) groups. Significant differences were also observed between high-fat and high-protein groups ($p = 0.009$). These findings indicate that diet substantially shapes both bacterial and fungal community structure.

Diet-Induced Changes in Gut Bacterial and Fungal Species At the phylum level, gut bacterial composition varied markedly with diet: Bacillota dominated the chow, high-carbohydrate, and high-fat groups (61%, 89%, and 77%, respectively), whereas Bacteroidota predominated in the high-protein and high-fiber groups (56% and 80%, respectively; Fig. 3A). At the order level, Lachnospirales predominated in chow, Erysipelotrichales and Oscillospirales in high-carbohydrate, and Peptostreptococcales-Tissierellales in the high-fat group,

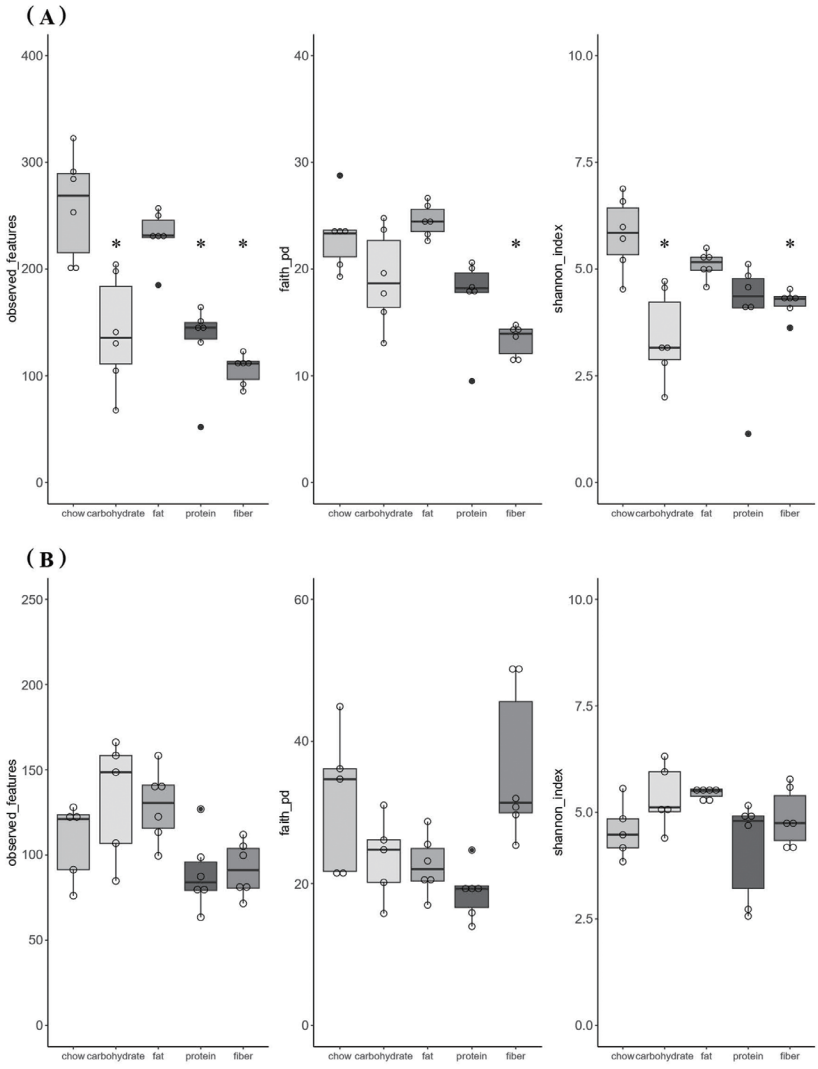


Fig. 1. Analysis of α-Diversity of The Gut Microbiota and Mycobiota under Different Diets, Using Observed Features, Faith's Phylogenetic Diversity, and Shannon Index

(A) Gut microbiome; (B) Gut mycobiome. Values represent the mean ± SD ($n = 6$ per group: chow, high-carbohydrate, high-fat, high-protein, and high-fiber). Significant differences were assessed using the nonparametric Kruskal–Wallis test. *$p < 0.05$, significant difference from chow by Steel's test.

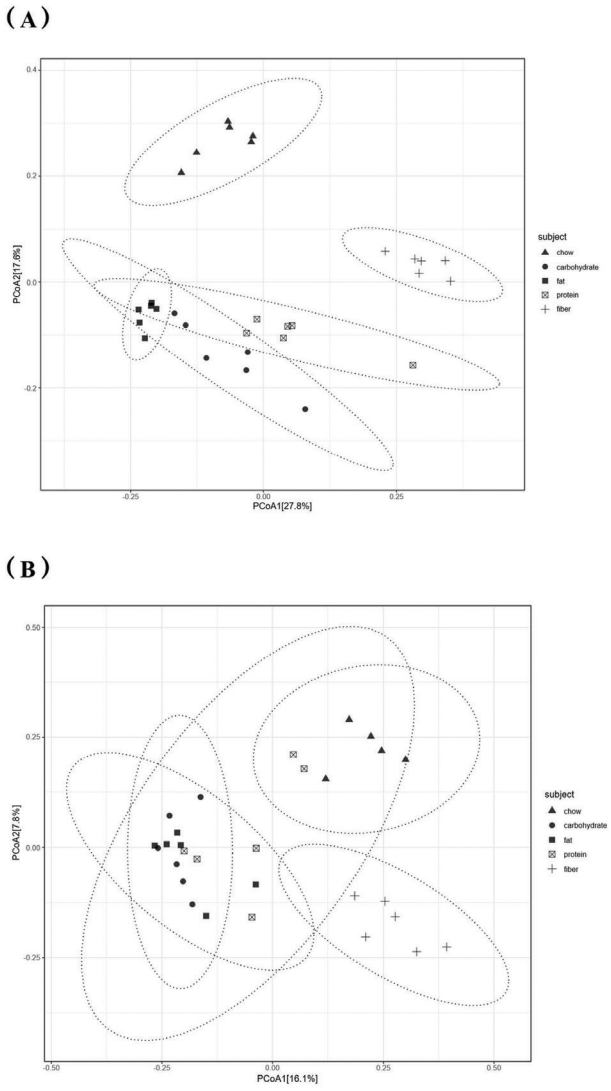


Fig. 2. Principal Coordinates Analysis (PCoA) of Gut Microbial Communities (n = 6 per group: chow, high-carbohydrate, high-fat, high-protein, and high-fiber)

(A) Gut microbiome; (B) Gut mycobiome. *P*-values were calculated using pairwise permutational multivariate analysis of variance (PERMANOVA).

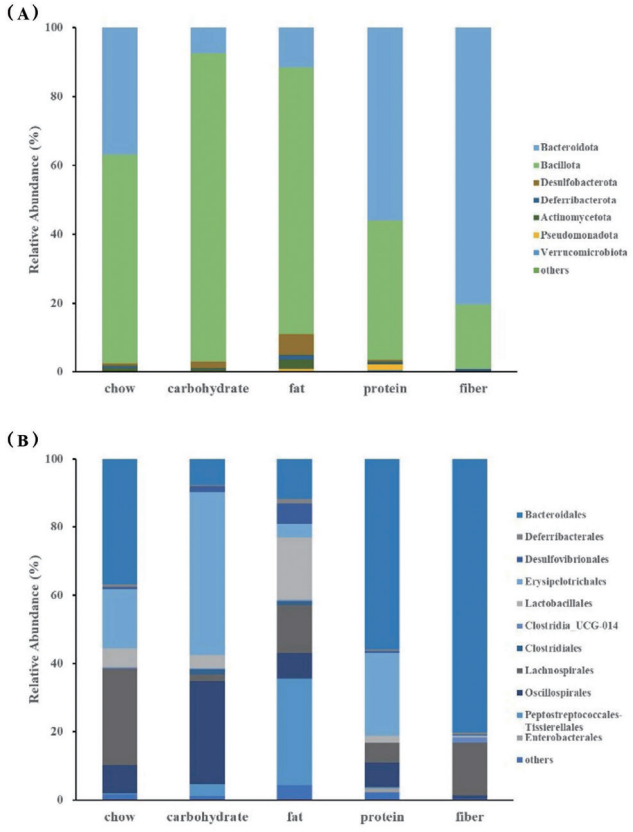


Fig. 3. Composition and Relative Abundance of The Gut Microbiome at The Phylum (A) and Order (B) Levels (mean of n = 6 per group: chow, high-carbohydrate, high-fat, high-protein, and high-fiber)

while Bacteroidales dominated both high-protein and high-fiber groups (Fig. 3B). LEfSe analysis revealed that the taxa Peptostreptococcaceae, Streptococcaceae, and Clostridiales were significantly enriched in the high-carbohydrate and high-fat groups compared with chow (Fig. 4A–B). Conversely, Muribaculaceae abundance was significantly higher in the chow group than in the high-carbohydrate and high-fat groups (Fig. 4B). In the high-protein and high-fiber groups, multiple bacterial taxa were significantly reduced relative to chow (Fig. 4C–D), consistent with the observed reductions in α-diversity.

At the phylum level, Ascomycota accounted for approximately 50% of fungi in chow, whereas Basidiomycota dominated the four purified diet groups (60–80%; Fig. 5A). At the order level, Eurotiales (~20%) and Saccharomycetales (~15%) predominated in chow but were markedly less abundant in other groups (Fig. 5B). Polyporales was detected in all groups and represented 33–40% of the community in the purified diets, making it the most abundant fungal order in these groups (Fig. 5B). LEfSe analysis of the fungal communities indicated that Aspergillaceae and Saccharomycetaceae were significantly enriched in chow compared with the other dietary groups (Fig. 6A–D). In contrast, Clavicipitaceae abundance was significantly increased in all dietary groups except the high-protein, relative to chow (Fig. 6A–D).

Relationship between Gut Bacterial and Fungal Families To examine interactions between gut bacteria and fungi, Spearman's rank correlation coefficients were calculated between the 22 bacterial families and 23 fungal families that met the relative abundance criterion described above (Fig. 7A–E). The highest number of strong correlations ($|r| > 0.8$) occurred in the high-protein group (40 combinations) and the fewest in the high-fiber group (21). Conversely, noncorrelated pairs ($|r| < 0.3$) were most frequent in the high-carbohydrate group (238) and least frequent in the high-fiber group (179). Notable correlations included the gram-positive anaerobic Eggerthellaceae in the chow group, which exhibited strong associations with seven fungal families (Fig. 7A). Similarly, Muribaculaceae displayed strong correlations with four fungal families in chow (Fig. 7A). Overall, the chow and high-fiber groups showed more numerous strong bacterial–fungal correlations (Fig. 7A, E), whereas the high-fat group showed weaker associations, such as the absence of a strong correlation between Ruminococcaceae and Polyporaceae (Fig. 7C). In the high-protein group, strong negative correlations were more prevalent (Fig. 7D); for example, Aspergillaceae showed negative, though non-significant, correlations with Eggerthellace-

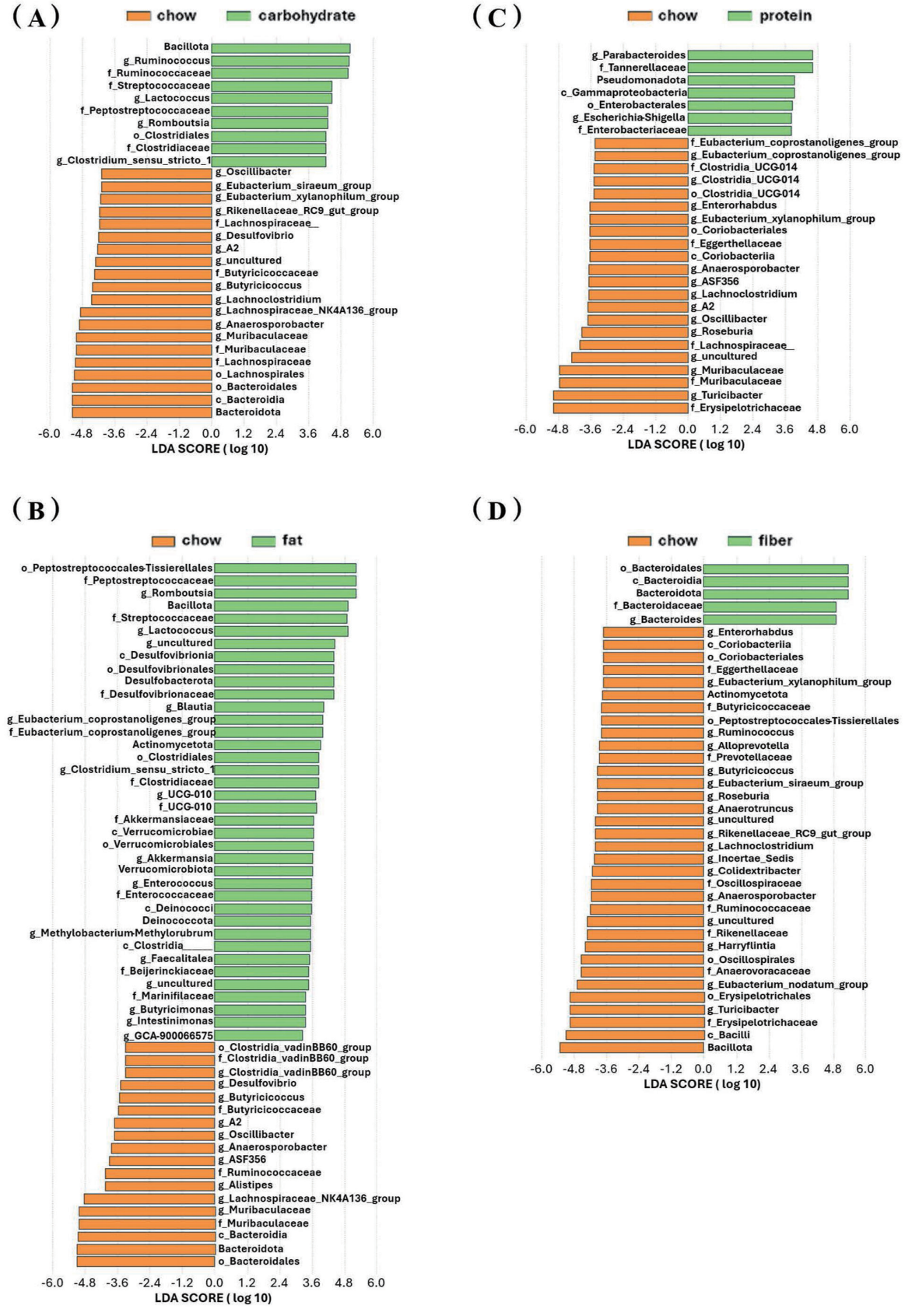


Fig. 4. Linear Discriminant Analysis Effect Size (LEfSe) Comparisons of Gut Microbiome Taxa

(A) chow vs. high-carbohydrate; (B) chow vs. high-fat; (C) chow vs. high-protein; (D) chow vs. high-fiber. Red indicates taxa enriched in chow; green indicates taxa enriched in the respective dietary group.

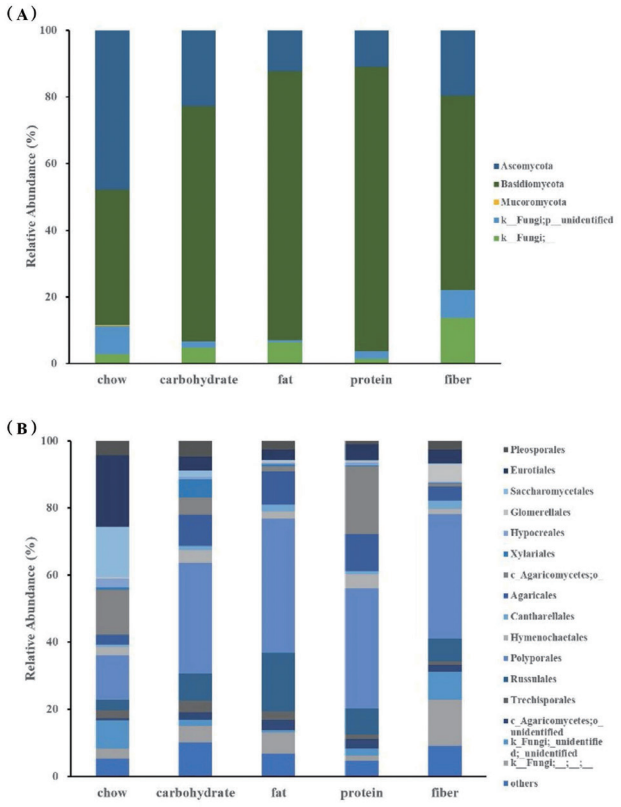


Fig. 5. Composition and Relative Abundance of the Gut Mycobiome at The Phylum (A) and Order (B) Levels (mean of $n = 6$ per group: chow, high-carbohydrate, high-fat, high-protein, and high-fiber).

ae in chow and Deferribacteraceae in the high-carbohydrate group (Fig. 7A, B), and Saccharomycetaceae showed significant negative correlations with Enterobacteriaceae, Peptostreptococcaceae, and Malasseziaceae in the high-protein group (Fig. 7D) and with Eggerthellaceae in the high-fiber group (Fig. 7E).

DISCUSSION

In the present study, we aimed to establish baseline data on diet-induced alterations in the gut microbiota and mycobiota, and their relationships. Our results showed that both α- and β-diversities of the gut microbiota varied depending on diet (Figs. 1A, 2A, and 3). Specifically, α-diversity of gut bacteria was significantly reduced in the high-fiber and high-protein dietary groups compared with chow. LEfSe analysis indicated that the number of significantly enriched bacterial taxa was more limited in these two groups than in the others (Fig. 4C, D). Notably, Peptostreptococcaceae and Streptococcaceae, which were enriched in the high-carbohydrate and/or high-fat diet groups in the present study, have previously been reported to be increased in inflammatory bowel disease, particularly in active ulcerative colitis.[31] However, these previously reported associations do not necessarily indicate a pro-inflammatory role of these taxa, and host inflammatory responses were not directly assessed in the present study. The high-fiber and high-protein diets were based on a high-carbohydrate (normal) diet supplemented with inulin or egg white, respectively (Supplementary Table 1). Yin *et al*. reported that individuals with a high genus *Bacteroides*/*Bifidobacterium* (Ba/Bi) ratio prior to inulin intake were more likely to benefit from the prebiotic effects of inulin, and that a higher Ba/Bi ratio was associated with greater α-diversity.[32] In the present study, the high-fiber diet was based on the high-carbohydrate diet with additional inulin. Given the reported association between baseline Bacteroides abundance and the microbial response to inulin, differences in the underlying microbial community may have contributed to the reduced α-diversity observed in the high-fiber group. The mechanism underlying decreased α-diversity in the high-protein diet group remains unclear. However, similar findings have been reported by Blakeley-Ruiz *et al*., who demonstrated that egg white protein increases the abundance of *Bacteroides thetaiotaomicron* while decreasing that of *Clostridia*,[33] consistent with our observations. Yu *et al*. reported that egg white intake also increased Pseudomonadota abundance and lipopolysaccharide (LPS) levels.[34]

Although less studied than the gut microbiota, the gut mycobiota also plays important roles in host physiology. In healthy individuals, genera such as *Saccharomyces*, *Malassezia*, *Candida*, and *Cladosporium* are abundant.[13,35] The abundance of *Candida* and *Malassezia* varies between individuals depending on environmental and dietary factors.[20,36] Gut mycobiota are also involved in activating immune responses, [16,17]; for example, "rewilding" specific pathogen-free (SPF) mice under natural conditions increases gut mycobiota and enhances immune activity, including T-cell differentiation.[37] Immunoglobulin A (IgA) in the intestinal tract has been shown to regulate fungi,[38–40] highlighting that fungal–host interactions are biologically significant. In the present study, α-diversity of the gut mycobiota did not differ significantly across dietary groups, suggesting that fungal richness is less sensitive to dietary change than bacterial diversity (Fig. 1). However, fungal composition varied between chow and purified diets, which showed higher relative abundances of *Agaricales* and *Polyporales* (Fig. 5B), both members of the phylum *Basidiomycota*, capable of degrading recalcitrant substrates such as cellulose and lignin, consistent with previous reports in human and animal guts.[13,36] LEfSe analysis revealed that Aspergillaceae and Saccharomycetaceae were significantly more abundant in chow than in the other dietary groups (Figs. 5B and 6). The abundance of Aspergillaceae is known to increase with vegetarian diets,[41,42] while Saccharomycetaceae is commonly associated with wheat- or barley-based foods such as bread and beer.[18] The present observations are therefore consistent with previous findings. Regarding β-diversity, gut mycobiota in the chow and high-fiber groups formed distinct clusters (Fig. 2B), a trend similar to the gut bacterial communities, indicating that diet substantially affects the overall microbial ecosystem. Although the exact composition of chow is undisclosed, it includes fiber-rich ingredients such as soybean meal and bran, which, together with inulin in the high-fiber diet, likely contribute to shaping gut microbial diversity.

Several studies have investigated gut bacteria–fungi interactions,[43–48] often focusing on *Candida albicans*. In the present study, we analyzed the effect of dietary habits on bacteria–fungi interactions by examining 22 bacterial families and 23 fungal families selected based on their relative abundance. The high-fiber group showed predominantly positive bacterial–fungal correlations, whereas the high-fat and high-protein groups showed largely negative correlations.

This study demonstrated that gut bacterial and fungal com-

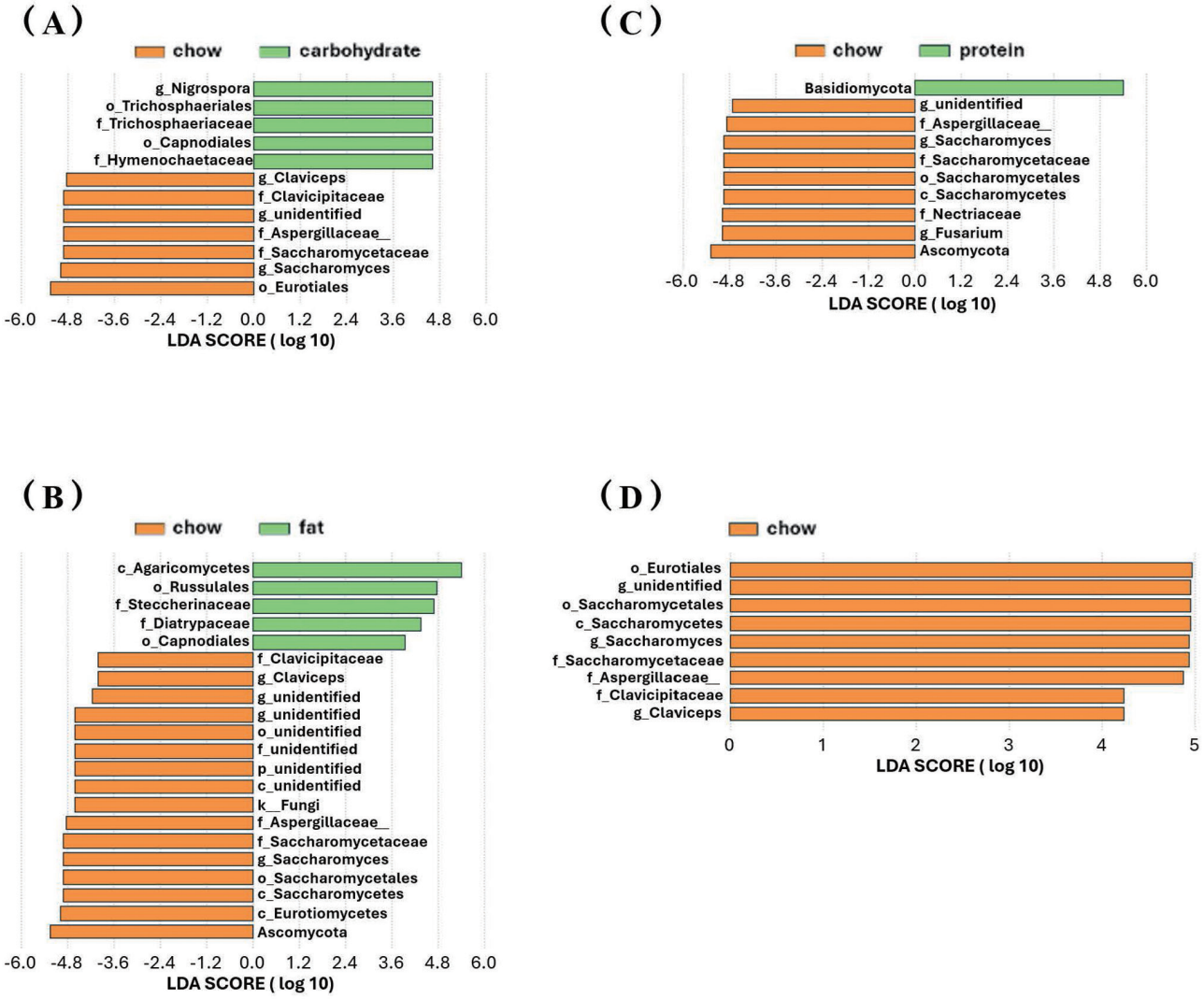


Fig. 6. Linear Discriminant Analysis Effect Size (LEfSe) Comparisons of Gut Mycobiome Taxa

(A) chow vs. high-carbohydrate; (B) chow vs. high-fat; (C) chow vs. high-protein; (D) chow vs. high-fiber. Red indicates taxa enriched in chow; green indicates taxa enriched in the respective dietary group.

munities vary according to dietary habits. Because diet is the primary energy source for gut microbes, it profoundly shapes microbial community structure; understanding the diet-associated state of experimental animals' gut microbiota is therefore important for translating preclinical research into clinical applications. Diet-induced alterations in the gut microbiota may also have implications for drug metabolism. Gut microorganisms possess diverse enzymatic activities capable of activating, inactivating, or otherwise modifying orally administered compounds. Importantly, diet can modulate such microbiome-mediated drug metabolism. For example, dietary factors have been shown to influence the microbial inactivation of digoxin by Eggerthella lenta, thereby affecting systemic drug exposure.[49] We have also previously demonstrated that dietary composition alters gut microbial metabolism of sennoside A and the laxative efficacy of Daiokanzoto.[11,12] These findings suggest that diet-dependent differences in microbial communities may represent one factor contributing to interindividual or interexperimental variability in drug responses. Previous studies have shown that commonly used drugs, such as metformin for diabetes,[50] nonsteroidal antiinflammatory drugs (NSAIDs),[51] and gastric acid secretion inhibitors[52] can alter gut bacterial community composition, as can traditional herbal medicines.[7–12] Pharmaceutical excipients, such as carboxymethyl cellulose,[53] hydroxypropyl methylcellulose,[54,55] and guar gum,[56] have also been reported to influence gut microbial communities, highlighting the importance of evaluating both the direct effects of drugs on the host and their indirect effects via the gut microbiota.

Several limitations should be considered. First, each dietary group was housed in a single cage, so diet and cage effects could not be separated; some observed differences may therefore reflect cage-specific effects in addition to dietary conditions, and future studies using multiple cages per group will be needed to distinguish them. Second, gut microbial communities differ substantially between mice and humans in taxonomic composition and relative abundance,[57,58] so diet-associated changes in individual taxa observed here should not be directly extrapolated to humans; rather, our findings provide baseline information on how dietary conditions can shape

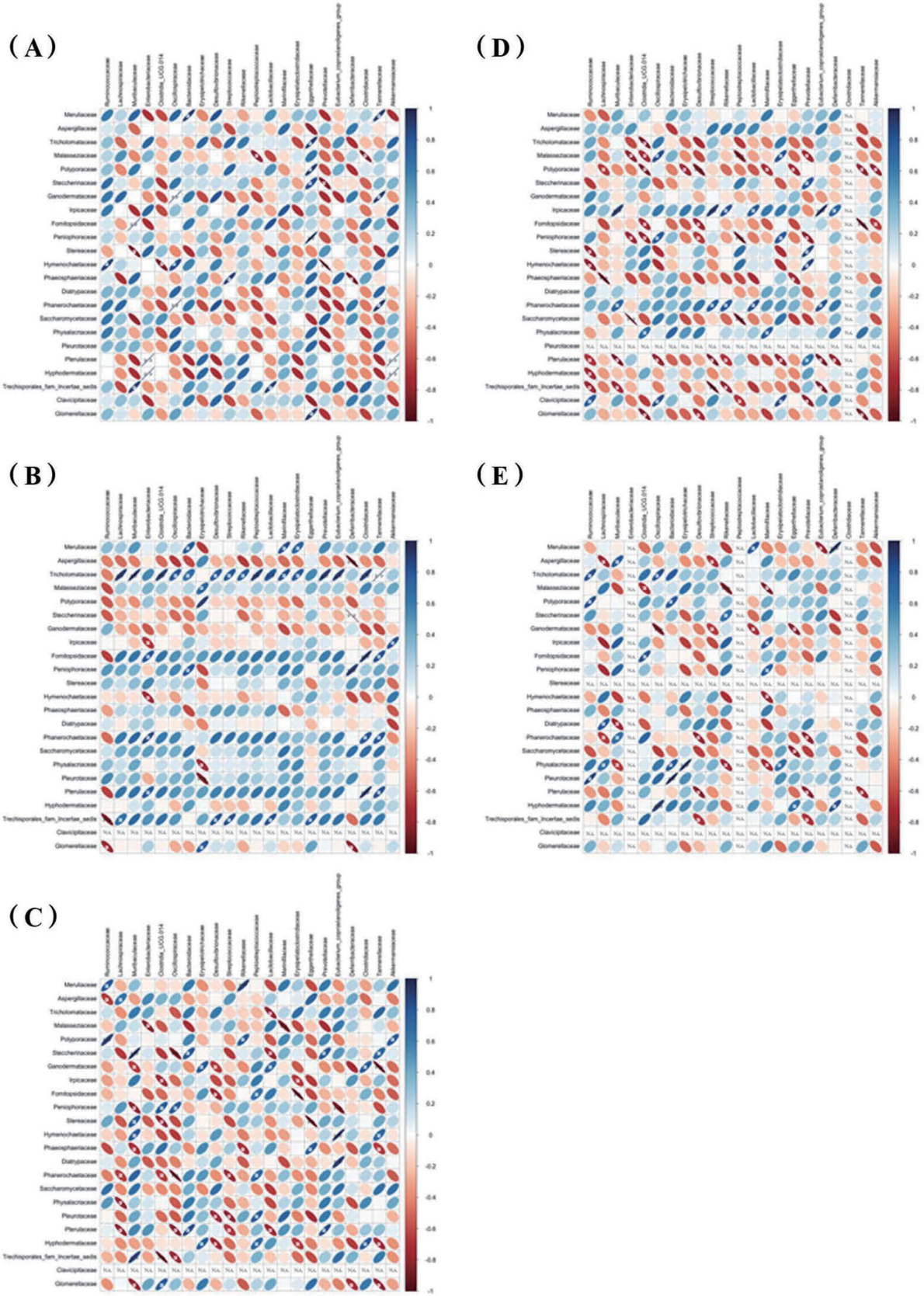


Fig. 7. Spearman's Rank Correlation Matrix of Dominant Gut Bacterial and Fungal Families in Each Diet

(A) chow; (B) high-carbohydrate; (C) high-fat; (D) high-protein; and (E) high-fiber. Strong correlations are represented by sharply angled ellipses, whereas weak correlations are represented by more circular ellipses. Color scale: 1 (dark blue) indicates a perfect positive correlation; -1 (dark red) indicates a perfect negative correlation. Families absent from a dietary group were excluded from correlation calculations (denoted as "NA").

the microbial background of mice used in preclinical studies. Third, this study was descriptive and did not directly examine whether these microbial differences affect drug metabolism, efficacy, or safety; the implications for drug responses discussed above should therefore be regarded as hypotheses rather than direct findings, and further studies combining dietary interventions with pharmacological assessments, as well as studies in humans, will be required to determine their translational relevance.

Acknowledgments The authors are grateful to Mr. Kazuki Tsukao for his technical assistance.

Conflicts of interest The authors declare that they have no conflicts of interest.

Supplementary materials This article contains the following Supplementary Materials.

Supplementary Table 1. Diet composition.

REFERENCES

1) Mitsuoka T. Establishment of intestinal bacteriology. *Biosci. Microbiota Food Health*, **33**, 99–116 (2014).
2) Sohail MU, Althani A, Anwar H, Rizzi R, Marei HE. Role of the gastrointestinal tract microbiome in the pathophysiology of diabetes mellitus. *J. Diabetes Res.*, **2017**, 9631435 (2017).
3) Ni J, Wu GD, Albenberg L, Tomov VT. Gut microbiota and IBD: causation or correlation? *Nat. Rev. Gastroenterol. Hepatol.*, **14**, 573–584 (2017).
4) Yachida S, Mizutani S, Shiroma H, Shiba S, Nakajima T, Sakamoto T, Watanabe H, Masuda K, Nishimoto Y, Kubo M, Hosoda F, Rokutan H, Matsumoto M, Takamaru H, Yamada M, Matsuda T, Iwasaki M, Yamaji T, Yachida T, Soga T, Kurokawa K, Toyoda A, Ogura Y, Hayashi T, Hatakeyama M, Nakagama H, Saito Y, Fukuda S, Shibata T, Yamada T. Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer. *Nat. Med.*, **25**, 968–976 (2019).
5) Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, Costea PI, Godneva A, Kalka IN, Bar N, Shilo S, Lador D, Vila AV, Zmora N, Pevsner-Fischer M, Israeli D, Kosower N, Malka G, Wolf BC, Avnit-Sagi T, Lotan-Pompan M, Weinberger A, Halpern Z, Carmi S, Fu J, Wijmenga C, Zhernakova A, Elinav E, Segal E. Environment dominates over host genetics in shaping human gut microbiota. *Nature*, **555**, 210–215 (2018).
6) Nagata N, Nishijima S, Miyoshi-Akiyama T, Kojima Y, Kimura M, Aoki R, Ohsugi M, Ueki K, Miki K, Iwata E, Hayakawa K, Ohmagari N, Oka S, Mizokami M, Itoi T, Kawai T, Uemura N, Hattori M. Population-level metagenomics uncovers distinct effects of multiple medications on the human gut microbiome. *Gastroenterology*, **163**, 1038–1052 (2022).
7) Takayama K, Matsui E, Kobayashi T, Inoue H, Tsuruta Y, Okamura N. High-performance liquid chromatographic determination and metabolic study of sennoside A in daiokanzoto by mouse intestinal bacteria. *Chem. Pharm. Bull. (Tokyo)*, **59**, 1106–1109 (2011).
8) Matsui E, Takayama K, Sato E, Okamura N. The influence of glycyrrhiza and antibiotics on the purgative action of sennoside A from Daiokanzoto in mice. *Biol. Pharm. Bull.*, **34**, 1438–1442 (2011).
9) Takayama K, Tsutsumi H, Ishizu T, Okamura N. The influence of rhein 8-O-β-D-glucopyranoside on the purgative action of sennoside A from rhubarb in mice. *Biol. Pharm. Bull.*, **35**, 2204–2208 (2012).
10) Takayama K, Morita T, Tabuchi N, Fukunaga M, Okamura N. The effect of anthraquinones in daiokanzoto on increasing the synthesis of sennoside A-metabolic enzyme derived from bifidobacteria. *J. Trad. Med.*, **30**, 215–220 (2013).
11) Takayama K, Tabuchi N, Fukunaga M, Okamura N. Rhein 8-O-β-D-glucopyranoside elicited the purgative action of daiokanzoto (da-huang-gan-cao-tang), despite dysbiosis by ampicillin. *Biol. Pharm. Bull.*, **39**, 378–383 (2016).
12) Takayama K, Takahara C, Tabuchi N, Okamura N. Daiokanzoto (Da-Huang-Gan-Cao-Tang) is an effective laxative in gut microbiota associated with constipation. *Sci. Rep.*, **9**, 3833 (2019).
13) Nash AK, Auchtung TA, Wong MC, Smith DP, Gesell JR, Ross MC, Stewart CJ, Metcalf GA, Muzny DM, Gibbs RA, Ajami NJ, Petrosino JF. The gut mycobiome of the Human Microbiome Project healthy cohort. *Microbiome*, **5**, 153 (2017).
14) Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, *et al*.; MetaHIT Consortium. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, **464**, 59–65 (2010).
15) Gillevet PM, Sikaroodi M, Torzilli AP. Analyzing salt-marsh fungal diversity: comparing ARISA fingerprinting with clone sequencing and Pyrosequencing. *Fungal Ecol.*, **2**, 160–167 (2009).
16) Kombrink A, Tayyrov A, Essig A, Stöckli M, Micheller S, Hintze J, van Heuvel Y, Dürig N, Lin CW, Kallio PT, Aebi M, Künzler M. Induction of antibacterial proteins and peptides in the coprophilous mushroom Coprinopsis cinerea in response to bacteria. *ISME J.*, **13**, 588–602 (2019).
17) Leonardi I, Gao IH, Lin WY, Allen M, Li XV, Fiers WD, De Celie MB, Putzel GG, Yantiss RK, Johncilla M, Colak D, Iliev ID. Mucosal fungi promote gut barrier function and social behavior via Type 17 immunity. *Cell*, **185**, 831–846.e14 (2022).
18) Lee EH, Kim H, Koh JH, Cha KH, Lee KK, Kim WU, Pan CH, Lee YH. Dysbiotic but nonpathogenic shift in the fecal mycobiota of patients with rheumatoid arthritis. *Gut Microbes*, **14**, 2149020 (2022).
19) Zuo T, Wong SH, Cheung CP, Lam K, Lui R, Cheung K, Zhang F, Tang W, Ching JYL, Wu JCY, Chan PKS, Sung JJY, Yu J, Chan FKL, Ng SC. Gut fungal dysbiosis correlates with reduced efficacy of fecal microbiota transplantation in Clostridium difficile infection. *Nat. Commun.*, **9**, 3663 (2018).
20) Shah S, Locca A, Dorsett Y, Cantoni C, Ghezzi L, Lin Q, Bokoliya S, Panier H, Suther C, Gormley M, Liu Y, Evans E, Mikesell R, Obert K, Salter A, Cross AH, Tarr PI, Lovett-Racke A, Piccio L, Zhou Y. Alterations of the gut mycobiome in patients with MS. *EBioMedicine*, **71**, 103557 (2021).
21) Tuck CJ, De Palma G, Takami K, Brant B, Caminero A, Reed DE, Muir JG, Gibson PR, Winterborn A, Verdu EF, Bercik P, Vanner S. Nutritional profile of rodent diets impacts experimental reproducibility in microbiome preclinical research. *Sci. Rep.*, **10**, 17784 (2020).
22) Heisel T, Montassier E, Johnson A, Al-Ghalith G, Lin YW, Wei LN, Knights D, Gale CA. High-fat diet changes fungal microbiomes and interkingdom relationships in the murine gut. *mSphere*, **2**, e00351–e17 (2017).
23) Gupta Y, Ernst AL, Vorobyev A, Beltsiou F, Zillikens D, Bieber K, Sanna-Cherchi S, Christiano AM, Sadik CD, Ludwig RJ, Sezin T. Impact of diet and host genetics on the murine intestinal mycobiome. *Nat. Commun.*, **14**, 834 (2023).
24) Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, *et al*. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.*, **37**, 852–857 (2019).
25) Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. J.*, **17**, 10–12 (2011).
26) Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA,

Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods*, **13**, 581–583 (2016).
27) Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.*, **41**, D590–D596 (2013).
28) Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Dueñas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Lücking R, Martín MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U, Peterson M, Põldmaa K, Saag L, Saar I, Schüßler A, Scott JA, Senés C, Smith ME, Suija A, Taylor DL, Telleria MT, Weiss M, Larsson KH. Towards a unified paradigm for sequence-based identification of fungi. *Mol. Ecol.*, **22**, 5271–5277 (2013).
29) *Core team. _. R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria (2024).
30) Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. Metagenomic biomarker discovery and explanation. *Genome Biol.*, **12**, R60 (2011).
31) Jalanka J, Cheng J, Hiippala K, Ritari J, Salojärvi J, Ruuska T, Kalliomäki M, Satokari R. Colonic Mucosal Microbiota and Association of Bacterial Taxa with the Expression of Host Antimicrobial Peptides in Pediatric Ulcerative Colitis. *Int. J. Mol. Sci.*, **21**, 6044 (2020).
32) Yin P, Yi S, Du T, Zhang C, Yu L, Tian F, Zhao J, Chen W, Zhai Q. Dynamic response of different types of gut microbiota to fructooligosaccharides and inulin. *Food Funct.*, **15**, 1402–1416 (2024).
33) Blakeley-Ruiz JA, Bartlett A, McMillan AS, Awan A, Vanhoy Walsh M, Meyerhoffer AK, Vintila S, Maier JL, Richie T, Theriot CM, Kleiner M. Dietary protein source alters gut microbiota composition and function. *bioRxiv* [Preprint], 2024.04.04.588169 (2025).
34) Yu H, Qiu N, Meng Y, Keast R. A comparative study of the modulation of the gut microbiota in rats by dietary intervention with different sources of egg-white proteins. *J. Sci. Food Agric.*, **100**, 3622–3629 (2020).
35) Hoffmann C, Dollive S, Grunberg S, Chen J, Li H, Wu GD, Lewis JD, Bushman FD. Archaea and fungi of the human gut microbiome: correlations with diet and bacterial residents. *PLoS One*, **8**, e66019 (2013).
36) Maas E, Penders J, Venema K. Fungal-bacterial interactions in the human gut of healthy individuals. *J. Fungi (Basel)*, **9**, 139 (2023).
37) Yeung F, Chen YH, Lin JD, Leung JM, McCauley C, Devlin JC, Hansen C, Cronkite A, Stephens Z, Drake-Dunn C, Fulmer Y, Shopsin B, Ruggles KV, Round JL, Loke P, Graham AL, Cadwell K. Altered immunity of laboratory mice in the natural environment is associated with fungal colonization. *Cell Host Microbe*, **27**, 809–822.e6 (2020).
38) Doron I, Leonardi I, Iliev ID. Profound mycobiome differences between segregated mouse colonies do not influence Th17 responses to a newly introduced gut fungal commensal. *Fungal Genet. Biol.*, **127**, 45–49 (2019).
39) Ost KS, O'Meara TR, Stephens WZ, Chiaro T, Zhou H, Penman J, Bell R, Catanzaro JR, Song D, Singh S, Call DH, Hwang-Wong E, Hanson KE, Valentine JF, Christensen KA, O'Connell RM, Cormack B, Ibrahim AS, Palm NW, Noble SM, Round JL. Adaptive immunity induces mutualism between commensal eukaryotes. *Nature*, **596**, 114–118 (2021).
40) Dambuza IM, Brown GD. Managing the mycobiota with IgA. *Nat. Microbiol.*, **6**, 1471–1472 (2021).
41) Hallen-Adams HE, Suhr MJ. Fungi in the healthy human gastrointestinal tract. *Virulence*, **8**, 352–358 (2017).
42) Suhr MJ, Banjara N, Hallen-Adams HE. Sequence-based methods for detecting and evaluating the human gut mycobiome. *Lett. Appl. Microbiol.*, **62**, 209–215 (2016).
43) Demir M, Lang S, Hartmann P, Duan Y, Martin A, Miyamoto Y, Bondareva M, Zhang X, Wang Y, Kasper P, Bang C, Roderburg C, Tacke F, Steffen HM, Goeser T, Kruglov A, Eckmann L, Stärkel P, Fouts DE, Schnabl B. The fecal mycobiome in non-alcoholic fatty liver disease. *J. Hepatol.*, **76**, 788–799 (2022).
44) Krüger W, Vielreicher S, Kapitan M, Jacobsen ID, Niemiec MJ. Fungal-bacterial interactions in health and disease. *Pathogens*, **8**, 70 (2019).
45) Noverr MC, Huffnagle GB. Regulation of Candida albicans morphogenesis by fatty acid metabolites. *Infect. Immun.*, **72**, 6206–6210 (2004).
46) García C, Tebbji F, Daigneault M, Liu NN, Köhler JR, Allen-Vercoe E, Sellam A. The human gut microbial metabolome modulates fungal growth via the TOR signaling pathway. *mSphere*, **2**, e00555–e17 (2017).
47) Cottier F, Tan ASM, Chen J, Lum J, Zolezzi F, Poidinger M, Pavelka N. The transcriptional stress response of Candida albicans to weak organic acids. *G3 (Bethesda)*, **5**, 497–505 (2015).
48) Shareck J, Belhumeur P. Modulation of morphogenesis in Candida albicans by various small molecules. *Eukaryot. Cell*, **10**, 1004–1012 (2011).
49) Haiser HJ, Gootenberg DB, Chatman K, Sirasani G, Balskus EP, Turnbaugh PJ. Predicting and manipulating cardiac drug inactivation by the human gut bacterium *Eggerthella lenta. Science*, **341**, 295–298 (2013).
50) Wu H, Esteve E, Tremaroli V, Khan MT, Caesar R, Mannerås-Holm L, Ståhlman M, Olsson LM, Serino M, Planas-Fèlix M, Xifra G, Mercader JM, Torrents D, Burcelin R, Ricart W, Perkins R, Fernàndez-Real JM, Bäckhed F. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nat. Med.*, **23**, 850–858 (2017).
51) Maseda D, Ricciotti E. NSAID-gut microbiota interactions. *Front. Pharmacol.*, **11**, 1153 (2020).
52) Imhann F, Bonder MJ, Vich Vila A, Fu J, Mujagic Z, Vork L, Tigchelaar EF, Jankipersadsing SA, Cenit MC, Harmsen HJM, Dijkstra G, Franke L, Xavier RJ, Jonkers D, Wijmenga C, Weersma RK, Zhernakova A. Proton pump inhibitors affect the gut microbiome. *Gut*, **65**, 740–748 (2016).
53) Chassaing B, Koren O, Goodrich JK, Poole AC, Srinivasan S, Ley RE, Gewirtz AT. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nature*, **519**, 92–96 (2015).
54) Russell WR, Gratz SW, Duncan SH, Holtrop G, Ince J, Scobbie L, Duncan G, Johnstone AM, Lobley GE, Wallace RJ, Duthie GG, Flint HJ. High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. *Am. J. Clin. Nutr.*, **93**, 1062–1072 (2011).
55) Cox LM, Cho I, Young SA, Anderson WHK, Waters BJ, Hung SC, Gao Z, Mahana D, Bihan M, Alekseyenko AV, Methé BA, Blaser MJ. The nonfermentable dietary fiber hydroxypropyl methylcellulose modulates intestinal microbiota. *FASEB J.*, **27**, 692–702 (2013).
56) Paudel D, Nair DVT, Tian S, Hao F, Goand UK, Joseph G, Prodes E, Chai Z, Robert CEM, Chassaing B, Patterson AD, Singh V. Dietary fiber guar gum-induced shift in gut microbiota metabolism and intestinal immune activity enhances susceptibility to colonic inflammation. *Gut Microbes*, **16**, 2341457 (2024).
57) Nguyen TLA, Vieira-Silva S, Liston A, Raes J. How informative is the mouse for human gut microbiota research? *Dis. Model. Mech.*, **8**, 1–16 (2015).
58) Hugenholtz F, de Vos WM. Mouse models for human intestinal microbiota research: a critical evaluation. *Cell. Mol. Life Sci.*, **75**, 149–160 (2018).